ISSN: 1224-7626

ANALELE UNIVERSITĂŢII DIN ORADEA

Fascicula CHIMIE
XXIX (29) 2022

EDITURA UNIVERSITĂŢII DIN ORADEA

2022

Editorial Adress
University of Oradea, Chemistry Departament
Str. Universitatii, nr.1, 410087, Oradea, Bihor, România
General Information
ISSN: 1224-7626
Place of publishing: Oradea, Romania
Year of the first issue: 1995
Releasing frequency: 1 issue / year
Language: English
Abstracting/Indexing:

Chemical Abstracts Service-CAS Source Index (CASSI)

Academic Research Index- ResearchBiB

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INFLUENCE OF THE OPERATING VOLTAGE ON THE TITANIUM PASSIVATION PROCESS BY ANODIZING

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Abstract. *Preliminary studies on Ti6Al4V titanium alloy samples were performed in order to investigate the influence of the operating conditions of the anodizing process on the corrosion resistance and chromatic effect of the oxidized surface. The voltage of the power supply varied in the range of 10 – 30 V, and the duration of the electrochemical deposition was about 80-90 seconds. The thickness of the anodized layer was calculated taking into account an average specific density of titanium oxide. Regarding the current density, it decreased continuously in the first 20 seconds, depending on the value of the supply voltage, then remaining constant until the end of the electrolysis. As a result of the experimental results, a directly proportional increase of the amount of deposited oxide and, implicitly, of the thickness of the samples was observed, together with the increase of the power supply voltage. The direct relationship between the supply voltage, the thickness of the deposited film and the colour of the titanium oxide layer obtained was also observed.*

Keywords*: titanium, passivation, anodizing, operating voltage, colour durability*

1. Introduction

The electrochemical passivation (anodizing) can be defined as the electrochemical growth of a solid oxide film on the surface of a metallic/semi-metallic substrate, obtained by the anodic polarization of the substrate, in an electrochemical cell. During the process, the electrons migrate from the metal which can be anodized to the cathode by external circuit. This result is called the ionization of metal atoms on the surface of the anode. The latter reacts with oxygen-containing anions from the electrolyte and finally a solid film is formed, usually oxide. Once the first monolayer is formed on the substrate, the further increase of the oxide film requires that the metal cations, produced at the metal/film interface, to be able to react with the oxygen-containing anions. Hence, the increase of the oxide film is based on the transport of metals cations and/or O^{2-} anions across the film.

It is worth mentioning that not all metals can be anodized. The conditions for a metal to be suitable for anodization are as follows:

- the metal must be capable to form an oxide compound with oxy-anions from the solution
- the metal oxide should be essentially insoluble in the selected electrolyte
- the metal oxide must be a weak electronic conductor. [1]

The metals that can be anodized are mainly called “valve-metals”, like: Al, Ta, Ti, Mb, Ze, Hf, W. These transitional metals form stable solid-oxide films, which are good ionic conductors and weak electronic conductors.

One of the mainly advantages of electrochemical passivation by anodic oxidation is the extreme simplicity of the process. To accomplish this process a simple experimental setup is used, which contains only a few elements: metal working electrode as the anode, auxiliary electrode, power source and an electrically conductive electrolytic solution. [1]

Due to their good mechanical properties, lightness, high stiffness, low hardness, high temperature resistance and good corrosion resistance, the titanium and its alloys are used in many application fields:

medical implants, submarines and aeronautical installations, automotive and aerospace industry. Titanium presents high resistance against corrosion in many acidic environments due to the formation on surface of an oxide protective film. The formation mechanism of the protective film influences its nature and physical properties, such as corrosion and wear resistance. It is known that corrosion resistance of the anodic oxide film depends on morphology, thickness, chemical composition and crystallization process. [2, 3] An ultra-thin amorphous oxide film increases the resistance to corrosion. Although the oxide film can prevent the metal from oxidizing in corrosive environments, it measures only a few mm thick and it is not as effective. [4]

Moreover, the oxide film formed in air is composed both of titanium ions (Ti^{4+}, Ti^{3+}, Ti^{2+}) and metallic atoms Ti^0. [5] Thermodynamically, Ti^{4+} is more active than other multivalent titanium species. In the literature, the titanium oxide films exhibit either *p*- or *n*-type conductivity depending on their stoichiometry and the nature of the resulting defects. [6] As a result, it can be easily destroyed, leading to crevice and galvanic corrosion [7]. Thereby, in order to improve corrosion resistance of the titanium and its alloys, surface modification methods are extensively applied, such as plasma, ion implantation, anodic oxidation treatment, spraying etc. [8]. Among these different techniques, anodic oxidation treatment is one of the most important surface modification procedures regarding the operability, cost and reproducibility of the prepared film stoichiometry. [4]

The main electrochemical reactions are the following:

cathodic site:

$$4H^+ + 4e^- \rightarrow 2H_2 \quad (1)$$

anodic site:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (2)$$

$$Ti + O_2 \rightarrow TiO_2\ (oxidation) \quad (3)$$

During anodic oxidation, the process parameters (electrolyte nature, potential value, anode current density, temperature, and anodic oxidation time) highly affect the growth and performance of obtained protective film. Compared with other acid anodic oxidation processes, the one using sulfuric acid bath has the advantage of low cost, less electric consumption, and good film wear resistance and corrosion resistance. Concerning the influence of working voltage on film corrosion resistance, the studies on the growth and properties of anodic film in low potential values (less than 10V) has not been reported. [2]

One of the most important parameters that affects the oxide film properties is the anodic potential. The literature revealed that crystalline structure of the anodic films on titanium occurs at high potential values, while low potentials lead to an amorphous structure. The corrosion resistance of the layer increases together with its thickness and the crystalline structure of titanium oxide film is more stable that the amorphous one.

Nevertheless, most studies have proven that higher potential values lead to increased formation of micropores and roughness on the surface of Ti and its alloys. [9] To improve the oxide film corrosion resistance it is necessary to reduce its surface roughness and micropores. According to literature data, the properties of the anodic oxide films are considerably determined by the electrochemical process type, although the final anodic potential has a constant value. The electrochemical process of anodizing can be potentionstatic, galvanostatic or potentiodynamic mode conducted. In terms of potentiostatic mode, the anodic oxide film formed is thicker and more crystalline than the other two types. [3]

Additionally, titanium oxide has been shown to exhibit high resistance to corrosion in most acidic media, and no anodic activity has been observed for a range of applied voltage. Further, the titanium oxide can act as catalytic support for the reduction reaction, due to its affinity to the oxygen reaction. Due to its excellent electrochromic properties, TiO_2 is used in photovoltaic cells and sensors. [4]

In anodizing of titanium, a much thinner transparent oxide layer is formed on the metal and finally colours results, not from the oxide layer absorbing added dyes as in case of aluminium, but rather from the effect of the thin oxide layer interfering with wavelengths of the incident light. The applied voltage takes on different values in case of titanium anodizing to obtain a variety of useful colours in many industries. So, metalworkers in the arts have used titanium,

niobium and tantalum for iridescent colouring when electrochemically or thermally anodized. [10-12]

The colours generated by anodizing are due to interference conditions between the light reflected at the oxide-air interface and the portion of incident light which is refracted by the oxide and then reflected by the metal-oxide interface. As can be seen from Figure 1, the optic path of the light radiation covers a longer distance with respect to that reflected one by the oxide external surface. So, if the two light radiances leaving the surface happen to be in phase, their colour will be sharper, though in the contrary case the colour will be lighter. [6]

The appearance of the colour on the anodised surface is the results of this process and the hue depends on the spectral components of the incident light that are either lighter or sharper by interference and thus on the difference in optic path, namely on the oxide thickness. When the oxide thickness exceeds a few hundred nm, the interference colour is lost. [6]

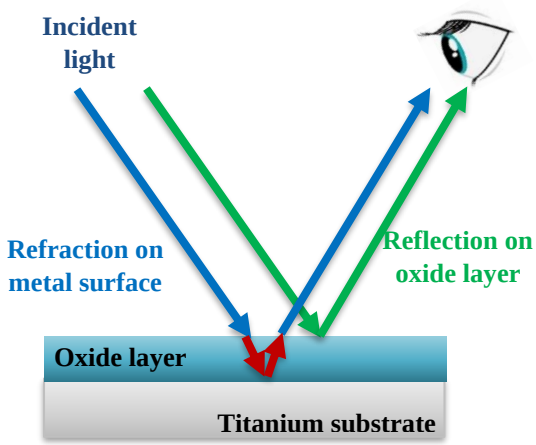

Figure 1. Interference of reflected light between titanium surface and the oxide film surface

In the present paper, preliminary studies on Ti6Al4V titanium alloy samples were performed in order to investigate the influence of the operating conditions of the anodizing process on the corrosion resistance and chromatic effect of the oxidized surface.

1. Experimental part

1.1. Materials

Preliminary cleaning of the metal samples surface was performed by polishing with fine abrasive paper, degreasing with sodium carbonate, and finally by rinsing with distilled water. To determine the thickness of the deposited titanium oxide, the metal samples were weighed before and after the electrochemical deposition.

The electrochemical oxidation experiments were performed at room temperature, in 0.5M H_2SO_4 aqueous solution, having a pH of 0.3, at an average current density of 400 $A \cdot m^{-2}$. The supply voltage varied in the range of 10-30 V, obtained from a stabilized DC power supply. The deposition time was about 80-90 seconds. The electrochemical cell used for the anodizing of titanium samples contains the Ti6Al4V titanium alloy working electrode, the negative porous graphite counter-electrode, and the saturated Ag/AgCl, KCl system as reference electrode, respectively.

The micromorphology of the samples was observed using scanning electron microscopy (SEM, TESCAN VEGA 3, Czech Republic) at an acceleration voltage of 30 kV.

2. Results and discussions

The experimental data and the calculated results on anodized titanium samples at different supply voltage values in acid aqueous electrolyte at room temperature are presented in Table 1. The thickness of the layer deposited by anodizing was calculated considering an average specific density of titanium oxide ($\rho = 4.37$ kg$\cdot$ m^{-3}).

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Table 1. *Experimental results on anodized titanium samples at different voltages in 0.5M H_2SO_4 aqueous solution at room temperature*

Ti6Al4V samples	Supply voltage, V	Active surface, $10^4 m^2$	Deposited amount, g	Layer thickness, mm
1	10	2.4	0.0012	1.1442
2	20	2.8	0.0016	1.3076
3	30	3.2	0.0021	1.5017

In terms of current density, it decreased continuously in the first 20 seconds from 375–500 $A \cdot m^{-2}$ to 6–8 $A \cdot m^{-2}$, depending on the value of the supply voltage, then remaining constant until the end of electrolysis.

The anodizing process may confer different colours to titanium oxide surfaces due to the interference between the light reflected at the oxide/air interface and the incident light part refracted by the transparent oxide film and then reflected by the metal/oxide interface. The result of this process is the appearance of different colours on the anodized surface [2, 3].

The shades generated depend on the spectral components of the incident light that are intensified or not by the interference, and thus on the difference of the optical paths due to the film thickness. [2, 3]. In this experiment, the colours of the anodized samples ranged from bronze to purple and light blue, respectively.

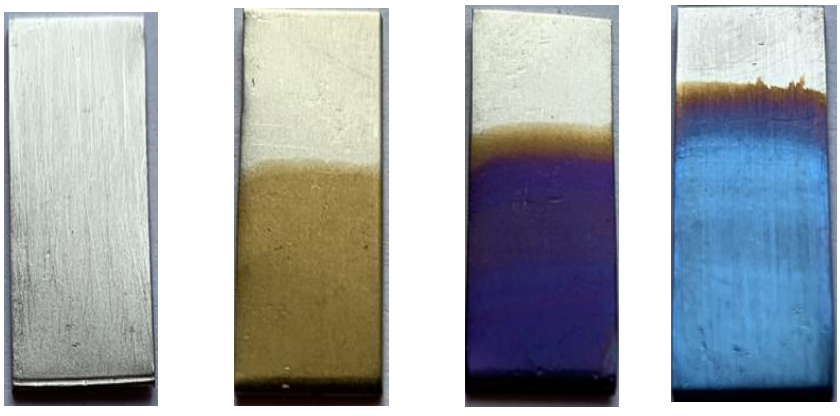
2.1. Influence of the operating voltage

To obtain interference colours with adequate intensity, the anodizing process contains 3 steps:

1) for a homogeneous surface, pickling of the metal surface in a solution of HF and HNO_3 acid (5% and 20%);
2) for a uniform final colour, the pre-anodizing step that takes place by immersing the titanium samples in a dilute solution of HCl acid and applying a supply voltage of 4 V;
3) the pre-anodized metal is anodized in a dilute electrolytic solution of H_2SO_4.

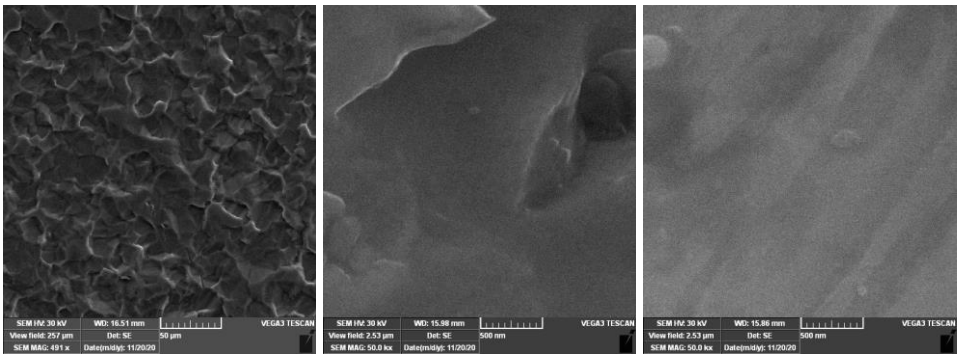
In Figure 2 the colour variations of the obtained TiO_2 film depending on the thickness and the applied voltage value in 0.5M H_2SO_4 aqueous solution, at room temperature, are presented.

The voltage applied at this stage will influence the colour shade, since it will determine the final thickness of the anodic oxide layer. In pre-anodizing process, the application of an exact voltage is strictly necessary, because different values that are too small or too high can lead to irregularities in the growth of the oxide on the surface, which will greatly decrease the colour saturation.



a) b) c) d)

Figure 2. *The colour variation of the TiO_2 film depending on the thickness and the applied voltage value in 0.5M H_2SO_4 aqueous solution at room temperature: a) 0 V; b) 10 V; c) 20 V; d) 30 V.*



a) b) c)

Figure 3. *Scanning electron microscopy (SEM) images of the surface of bare titanium (a) compared with the titanium oxide film formed at different applied voltages: (b) 10 V; (c) 30 V.*

In case the coloured layer is accidentally scratched or damaged, the same anodizing process was repeated after a second pickling stage applied on already anodized samples. For all samples and under similar conditions, the anodizing process was repeated with the same volume of solution to check the amount of titanium that could be anodized before the need for electrolyte refresh, and whether the electrolyte consumption would affect the process.

Figure 3 shows several SEM micrographs of the bare titanium surface compared with the titanium oxide film formed at different applied voltages. The passive film formed on titanium surface is relatively dense, some of the micropores are slightly smaller. When the anodic potential continued to increase, the structures composed of crystalline grains grow intensively and spread, covering the film surface and the passive film becomes flat and dense.

The titanium anodizing process has applications in the medical device and aerospace industries because it gives mechanical strength to the parts and allows easier identification depending on the assigned colour. For aesthetic reasons, this method has applications in the case of dental implants, and more precisely for prosthetic abutments, because they can give a grey metallic hue to the implants. Recently, another use has emerged in the jewellery industry due to obtaining very durable colours, shades and combinations, which cannot be obtained by other methods.

CONCLUSIONS

Titanium passivation is a simple, inexpensive and fast process that can be easily accomplished, presenting versatility in different industries.

The colouring process can be controlled by modifying the value of the applied voltage and hence the transparent oxide layer, thereby obtaining a range of colours. Colouring is a clean process because it does not involve the use of paints and does not produce waste. The final products are biocompatible and have no residues.

The anodic oxide film with rich colours and smooth surface was obtained by using 0.5M sulfuric acid as the electrolyte on the surface of titanium alloy. The obtained anodized samples were coloured and easily distinguishable depending on their anodized voltage. The current intensity recorded during the anodization process presented an abrupt drop off with time due to the formation of a non-conductive protective layer.

Titanium oxide film has been formed at the initial stage of anodic oxidation, by the comprehensive effect of oxide growth and dissolution. The corrosion resistance of titanium increases after anodic oxidation and titanium is more difficult to destroy with the increase of the oxidation potential. It is found that the thickness of the formed films increased with increasing applied voltage.

ACKNOWLEDGMENTS

The author Ioana-Maria Nicola is enrolled as PhD student, with tuitions fees supported by the Romanian Ministry of Education.

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Analele Universităţii din Oradea-Fascicula Chimie, Vol. XXIX (29), p.11-15, 2022

EVALUATION OF HEMIC AND NON-HEMIC Fe CONTENT IN DIFFERENT KIND OF CHICKEN EGG YOLK

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Abstract. *The content of heme and non-heme bonded iron, existing in the protein composition of the egg yolk, was investigated by the spectrophotometric method using o-phenanthroline. Grange eggs and eggs from industrial farms source, both brown-shelled and white-shelled lots were investigated. The yolks were separated in lipidoprotein granules and serum (liquid phase) and then the Fe^{+2} content was measured using spectrophotemetric method with o-phenanthroline. The Fe^{+2} contained in the lipidoproteicgranules and serum yolk phases was compared based on the type of egg.*

Key words: Spectrofotometric determination, egg yolk, o-phenanthroline, Fe^{+2}

INTRODUCTION

The egg yolk contains 2 major proteins that bind iron: phosvitin contained in the yolk granules and ferritin - in the yolk serum [1].

Ferritin is the main form of protein with the role of storing Fe in the cells of almost all types of organisms (animals, higher plants, algae and bacteria). Fe storage is in the form of ferrous hydroxide, a non-toxic soluble form of Fe [2].

Phosvitin is a highly phosphorylated, globular glycoprotein that has a strong metal binding capacity. It is composed of α- and β-phosvitin. Phosvitin has a specific composition of amino acids where serine predominates, which is mostly phosphorylated. In general, phosvitin consists of 217 amino acid residues, the basic region including a chain of 99 amino acids, the others being grouped in 14 residues interspersed with arginine, lysine and asparagine. [3].

An egg consists of three parts: the shell, the white and the yolk. The chemical composition of an egg depends on several factors: species and breed, the type of diet or the age of the egg.

The shell represents approximately 10% of the weight of an egg and is composed of calcium carbonates (94%), magnesium (1.5%), phosphates and other organic substances insoluble in water.

The egg white represents approximately 55% of the total weight of an egg. 85% of the egg white is represented by water, 12% protein substances (ovalbumin), and lipids and carbohydrates represent less than 1% of the weight of the egg white. Mineral salts (potassium, sodium, calcium) and vitamins are found in small quantities in the egg white.

The yolk represents approximately 35% of the weight of the egg, the dry matter being approximately 50-52%, depending on the age of the hen and the duration of storage. The yolk contains all the resources necessary for the development of the new organism: nitrogenous substances, lipids, vitamins or mineral salts. Proteins represent 16%, lipids 32% (triglycerides and lecithin with the highest weight), and the most important mineral elements in the yolk are phosphorus, calcium, iron and a series of trace elements. The lipid:protein ratio is 2:1, the lipids in the yolk being exclusively present in lipoprotein-type associations.

The yolk is a complex system, structured on different levels that consist of aggregates (granules) in suspension, in a yellow, translucent fluid (plasma) containing lipoproteins and proteins. These granules consist of circular shaped complexes with a diameter between 0.3 and 2 μm. [4]

MATERIAL AND METHOD

Investigates egg samples

Two lots of domestically produced eggs purchased from the producer (from cicken raised on the ground in grange) and two lots of eggs purchased from the supermarket (from industrial farms) were

investigated. Both brown-shelled and white-shelled lots eggs were investigated. Eggs with approximately the same volume were chosen. The term of keeping the eggs did not exceed 7 days.

Preparation of samples

The yolk was separated into two fractions after two-fold dilution with 0.3 M NaCl and centrifugation at 10,000 g (30 min), the dark orange supernatant representing the plasma fraction, while the translucent pellets are the granules. They represent 22% of the yolk's dry matter, containing 50% of the yolk's proteins and 7% of its lipids, being composed of HDL (high-density lipoproteins) (70%) and a protein - phosvitin - (16%), found in connection with the phosphocalcium bridges between the phosphate groups of the phosphoseryl residues. Some of the LDL (low-density lipoproteins) are included in this granular structure.

The acidification and alkalinization causes the destruction of the structure of the granules and the solubilization of their constituents, by increasing the number of positive (NH_4^+) or negative (COO^-) charges. The plasma portion of the yolk contains 78% of the dry matter of the yolk, and the aqueous phase in which the particles are in suspension. This comprises 90% of the yolk's lipids (of which 70% triglycerides, 25% phospholipids and 5% cholesterol) (including almost all carotenoids) and 50% of its proteins. [4]

The characteristics of the studied eggs

The eggs selected to be analyzed were broken and the masses of the three components were measured using a digital balance.

The separation of the two components from the egg yolk was performed according to the method presented by Laca A. [5,6].

Egg yolk represents an emulsion, which can be relatively easily fragmented into lipidoprotein granules and liquid phase - egg yolk serum.

Initially, the egg yolk was treated with NaCl, 5% and NaOH, 0.2N to reduce the bonds between proteins and fats in lipoproteins, then the mixture was treated with pectin gel extracted from citrus (with an increased content of CH_3COO^- groups), which possesses hydrophilic qualities. Treating egg yolk samples with NaCl and NaOH solutions produces an intense hydration of proteins, accelerates the separation of the fraction of organic macrocompounds - proteins and lipids from the liquid phase of the yolk. Subsequent treatment with pectin gel favors the clarification of the yolk serum (it immobilizes proteins and fats contributing to the formation of yolk granules). The effect of separating the granules fixed with serum pectin gel was obtained by centrifugation at high rotations and low temperatures of the sample (4ºC). After centrifugation for 30 min. (7000 rpm, for 20 min.) the serum was separated from the egg yolk and respectively the granules (translucent pellets).

The pectin gel was prepared from pectin purchased online from Balance food. A 2% pectin solution prepared with distilled water was used.

After treating the egg yolk and centrifugation, the two phases were separated

Table. 1 Sampled eggs characterisation

No. sample	Egg sample	Whole egg (g)	Egg white (g)	Egg yolk (g)	Egg shell (g)
1	White shell household egg	63,43	34,75	26,63	8,12
2	Brown shell household egg	62,56	35,76	28,07	7,69
3	White shell industrial producer egg	68,71	38,65	29,39	9,26
4	Brown shell industrial producer egg	66,32	35,89	27,55	8,34

Table 2. Processing of yolk samples to separate the two fractions; lipidoprotein granules and serum (liquid phase)

no. sample	Yeld g	NaCl 5% (mL)	NaOH 0,2 N (mL)	pectin gel 2 %
1	26,63	4	1	10
2	28,07	4	1	10
3	29,39	4	1	10
4	27,55	4	1	10

Table 3. Obtained two egg yolk fractions (lipidoprotein granules and serum-liquid phase)

no. sample	Amount of lipidoprotein granules g / %	The amount of serum g / %
1	19,35 / 35%	35,69 / 65%
2	23,02 / 42%	31,81 / 58%
3	18,98 /32%	40,35 / 68%
4	21,95 / 38%	35,83 / 62%

It is observed that following the separation, between 32%-42% lipidoprotein granules and 58%-68% serum (liquid phase) were obtained from the yolks of the analyzed eggs, in good agreement with the data from the literature. In the literature [6], it is mentioned that on average, 30-45 g of lipidoprotein granules and 40-60 g of yolk serum are obtained from 100 g of egg yolk.

Principle of Fe^{+2} determination

The amount of iron in the granules and respectively in the egg yolk serum was determined by the spectrophotometric method after calcination.

The ash obtained during the calcination of the samples was dissolved in double-distilled water and acidified with concentrated sulfuric acid up to pH=4. The solution obtained was treated with 1,10-phenanthroline and the intensity of the red color, corresponding to the concentration of the Fe complex, was recorded as absorbance at λ= 510 nm.

Plotting the calibration curve

To plot the calibration curve, a series of standard solutions with concentrations of 1,3,5,7,9 ppm $Fe^{+2}$was prepared.

A Mohr's salt solution ($Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$) was used as a stock solution by dissolving 0.0702 g in a 100 mL volumetric flask, acidifying with 2.5 mL of concentrated sulfuric acid and adding double-distilled water sign.

The standard solutions were prepared by dissolving 1, 3, 5, 7, 9 mL of stock solution in 100 mL volumetric flasks, adding over each 5 mL of o-phenthroline solution (prepared by dissolving 100 mg of o-phenthroline in 100 mL bidistilled water) and 8 mL acetic acid/acetate buffer solution (65 mL 0.1 M acetic acid + 35 mL 0.1 M sodium acetate) and bringing them to the mark.

The blank solution was prepared without the addition of Fe^{+2} solution. The solutions are colored red depending on the concentration of Fe^{+2}. After 5 minutes, when the red color of the complex formed between o-phenanthroline and Fe^{+2} reaches its maximum, the absorbance curves are recorded with the UV-VIS spetrophotometer at 510 nm.

Table 4. Preparation of standard solutions with known Fe^{+2} concentrations.

Fe^{+2} ppm	Mohr's salt stock solution mL	o-phenantrolin solution mL	Acetic acid/acetate buffer solution mL
0	0	5	8
1	1	5	8
3	3	5	8
5	5	5	8
7	7	5	8
9	9	5	8

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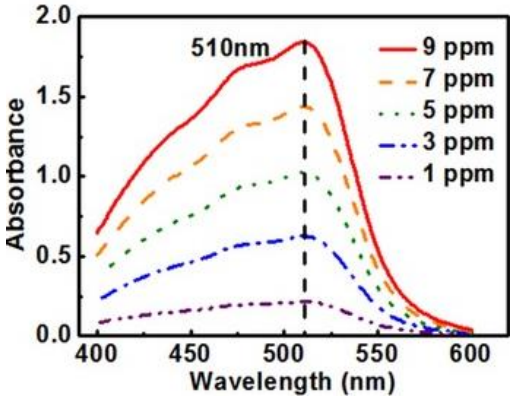

Figure 1. Absorbance curves recorded for standard Fe^{+2} solutions

The standard curve of the absorbances obtained at 510 nm (which correspond to the maximum peak) depending on the concentration of Fe^{+2}.

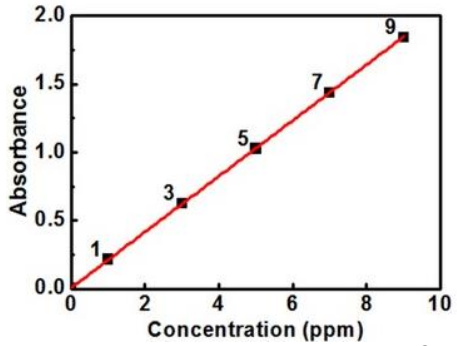

Figure 2. Calibration curve for Fe^{+2}

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Calibration curve is described by equation: y=0,99114x+0,00701

Sample preparation for Fe^{+2} content measurement

The egg yolk samples were calcined. The ash obtained was dissolved in a 100 mL volumetric flask and acidified with concentrated sulfuric acid to pH 4.

From the dissolved samples, 87 mL were taken to which 5 mL of o-phentroline solution and 8 mL of acetic acid/acetate buffer solution were added.

After 5 minutes the absorbances were measured. From the absorbance value with the help of the calibration curve, the amounts of Fe^{+2} contained in the analyzed samples were calculated.

RESULTS INTERPRETATION

The obtained results for Fe^{+2} determination in the yolk fractions are presented in table 5.

Table 5.

no. sample	Egg sample	Absorbance	Fe^{+2} ppb	Fe^{+2} µg/100g yolk fraction	Fe^{+2} µg/100g yolk
1	White shell household egg-Serum	4,44	3,83	270	460,47
	White shell household egg-lipidoprotein granules	3,13	2,7	190,47	
2	Brown shell household egg Serum	3,52	4,1	289,00	493,42
	Brown shell household egg lipidoprotein granules	3,36	2,9	204,42	
3	White shell industrial producer egg -Serum	4,10	3,54	249,53	421,53
	White shell industrial producer-lipidoprotein granules	2,83	2,44	172.00	
4	Brown shell industrial producer egg-Serum	4,36	3,76	265,04	445,49
	Brown shell industrial producer egg -lipidoprotein granules	2.96	2,56	180,45	

CONCLUSIONS

The yolk of eggs from the local producer (raised on the ground in the ground) have a higher Fe^{+2} content than the eggs purchased from the industrial producer.

Brown-shelled eggs have a higher yolk Fe^{+2} content than white-shelled eggs, regardless of their source

The serum (liquid fraction of the yolk) has a higher Fe^{+2} content than the phosphoprotein fraction

The phosvitin protein contained in the yolk lipidoprotein granules and the ferritin in the composition of the egg yolk serum are sources with an increased potential of easily assimilable iron.

The whole egg yolk or the two fractions (lipidoprotein granules and respectively serum), lyophilized to be preserved, could be used as sources of organic iron in the pharmaceutical industry.

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THE STUDY OF THE PROPERTIES AND RESISTANCE TO DEGRADATION OF MILK PROCESSED BY DIFFERENT TECHNIQUES

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Abstract. *In this paper we aimed to analyse and study the behaviour of 7 different varieties of milk, marketed in our region. They showed very similar parameters of pH, acidity and density, characteristics of fresh milk samples kept under appropriate conditions, during the shelf life mentioned by the manufacturer. The pH of the milk samples varied between 6.2 and 6.4, and the acidity varied between 1.7 °T for the 3.5% UHT milk and 2.4 °T for the 3.5% pasteurized milk. The small density variations in milk samples with 1.5% fat concentration compared to the same variety with 3.5% fat concentration did not follow a unique rule due to the complexity of the sample composition. Regarding milk casein concentration, the lowest concentrations were obtained for lactose-free milk, and the highest casein concentrations were obtained in samples with higher fat content, 3.5%. When storing milk samples for 4 days at room temperature, UHT milk proved to be the most stable. Heating the samples at different temperatures on the 4th day of storage at room temperature indicated that the 3.5% lactose-free milk was the most influenced.*

Key words: milk, casein, pH, acidity, density

INTRODUCTION

Milk is a beneficial food for people, especially for children and the elderly, due to its easy assimilation in the body. One litter of cow's milk having 4.8% lactose, 3.5% protein and 3.5% fat provides the body with an energy intake of 668 Kcal [1-3]. Milk consumption increases resistance to toxic substances and infections, prevents diseases, such as osteoporosis. The name given by nutritionists to milk is the "fountain of health" because it can supply the daily requirement of animal protein [4-6].

The first evidence of the production and processing of cow's milk was found in Mesopotamia from the Sumerian people and dates to 5000-4000 BC. In our country, following some archaeological discoveries southeast of Sarmisegetuza, some information about milk processing appears, but these are not very numerous [7-9].

Milk has over a hundred nutrients necessary for human life (20 amino acids, over 10 fatty acids, 4 types of lactose, 25 vitamins, over 45 mineral elements, proteins) [10-12]. Milk can be classified according to composition, according to quality, according to processing methods and from a hygienic point of view. According to food standards, commercial milk is of superior quality because the protein or carbohydrate content is not changed and not all vitamins are destroyed [13-15].

MATERIALS AND METHODS

In this paper, we aimed to determine different parameters for the characterization of some milk varieties marketed in our region, which differ one each other by composition (fat, lactose), by the source they come from (classic and organic products) and by the way in which the preservation, sterilization of the finished product was carried out (pasteurization or UHT).

Seven categories of samples from different manufacturers were taken in the study (Fig. 3):

1. Lactose-free milk, with 1.5% fat (semi-skimmed);
2. Lactose-free milk, with 3.0% fat (whole);
3. Pasteurized milk, with 1.5% fat (semi-skimmed);
4. Pasteurized milk, with 3.0% fat (whole);
5. UHT milk, with 1.5% fat (semi-skimmed);

6. UHT milk, with 3.0% fat (whole);
7. Pasteurized BIO milk, with 3.7-4.1% fat (whole);

For each of the samples, the pH, density, acidity, and amount of casein were determined. The obtained results were compared and discussed. For each milk sample, the pH variation over time, but also with increasing temperature, was followed. The results were recorded in evolution graphs and analyzed [15-20]. A Hanna Instruments portable pH meter was used for the study. Laboratory calibrated instruments and analytical purity reagents were used for all procedures performed.

Determination of pH

Both, the pH and acid-base balance values of milk at 25℃ normally vary within a narrow range of values from 6.5 to 6.8. In milk there are many components that function as a pH buffer, and the most important of these are caseins and phosphates [17]. The pH values obtained for the seven types of milk samples analysed are presented in Table 1.

Determination of acidity

Acidity is one of the important quality indicators that give information about the degree of freshness of milk. Acidity is decided by titrating 10 ml of milk with 0.1 N NaOH solution in the presence of phenolphthalein. In our country, acidity is expressed in degrees Thorner (°T) and is the number of millilitres of 0.1 N NaOH solution used to neutralize the acidity of 100 ml of milk. According to Romanian standards, the maximum acidity allowed for milk is 2.0°T, because a higher acidity would start protein coagulation during heat treatment [18]. The range of normal milk acidity values is between 13-20 mmol L^{-1}, which corresponds to 1.3-2.0 °T. The results obtained are recorded in Table 1.

Determination of density

Density is the most variable parameter of milk. The density of milk is given by the sum of the elements that make up the dry matter (SU) of milk given by proteins, sugars, mineral salts and less fat. The density varies inversely proportional to the fat content and in direct relation to the content of proteins, lactose and salts. The usual limits of milk density variation are between 1.027-1.033 g L^{-1} [23]. Density and milk fat are the quality parameters of milk. The milk of cows from the mountain area has a higher percentage of fat, but a lower density, compared to that from the plain area. The energetic balancing of the cows' daily feed in proteins, soluble sugars - fodder sugar beet, molasses and minerals leads to an increase in milk density [8,11,15, 23]. To find the density, 25 mL of milk from each sample were weighed on a balance at room temperature, and the results obtained can be seen in Table 1.

Determination of casein in milk

Casein (fig. 1) is the main protein of milk. Casein is a substance specific to milk secretion, representing about 80% of all substances with nitrogen atoms in milk. In cow's milk it is 2.5 - 3% of the total mass. In some pathological cases, a decrease in casein content was seen.

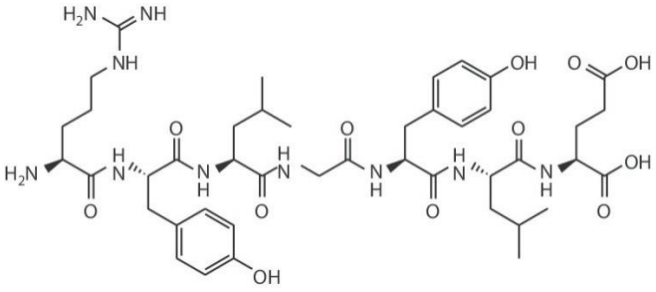

Fig. 1. Chemical structure of casein

The casein molecule has a complex structure. Casein is an acidic phosphoprotein that forms soluble salts with alkali metals and partially soluble with calcium. 18 different amino acids were highlighted, whereby a series of amino acids indispensable for the good functioning of the body and which cannot be synthesized by it, called essential amino acids such as: leucine, isoleucine, lysine, arginine, methionine, phenylalanine, histidine, tryptophan and valine. Casein has the property of coagulating in the presence of enzymes - chymosin, pepsin, in an acidic environment (pH 4.5-4.7). These properties are used in the milk processing industry in the form of cheeses or acidic preparations. In its

pure form, casein is presented as a white, tasteless and odourless powder [18-19].

Besides casein there are other proteins. β-Lactoglobulin is found in exceedingly lesser amounts but stands for 50% of whey. It has a higher sulphur content than casein and an extremely low phosphorus content. Its function is unknown, but it may be that of binding proteins to fatty acids or lipids. There might be a relationship between the presence of lactoglobulin and the transport of immunoglobulins after the formation of colostrum [16, 17]. α-Lactalbumin is 25% of the protein content of milk whey and is indispensable in the synthesis of lactose, in other words it engages in the control of milk secretion. It has a relatively low nitrogen content and a high sulphur content and binds calcium and zinc ions. It does not contain phosphorus, is soluble in water and precipitates under the action of heat (above 72°C) [18, 19]. Other proteins in lower concentrations are serum albumin, not synthesized in the mammary gland, whose concentration increases during mastitis or mammary involution; immunoglobulins (IgG_1, IgG_2, IgA, IgM), which are in high concentration in colostrum, but in low concentration in milk and are part of the mammary immune system, and lactoferrin, an iron-bound protein with antibacterial properties [15-17].

The dosage of casein is based on its acidic nature and consists in finding the excess of NaOH that remains after casein neutralization. Casein is separated by precipitation at pH 4.7 with acetic acid, followed by its passage into solution as sodium caseinate by treatment with 1 N NaOH. The excess NaOH added is titrated with 0.1 N H_2SO_4 in the presence of phenolphthalein indicator [19] The results obtained can be found in Table 1.

$$casein + NaOH \rightarrow sodium\ caseinate + NaOH_{exces}$$
$$2NaOH_{exces} + H_2SO_4 \rightarrow Na_2SO_4 + 2H_2O$$

Table 1. Values of pH, acidity, density and casein concentrations determined in milk samples

	Sample	pH	Acidity (°T)	Density ρ (gcm^{-3})	Casein (g %)
1	1,5% lactose-free milk	6,2	1,84	1,0320	3,466
2	3,5% lactose-free milk	6,3	1,67	1,0280	2,856
3	1,5% pasteurized milk	6,4	1,59	1,0260	10,176
4	3,5% pasteurized milk	6,3	2,00	1,0280	12,494
5	1,5% UHT milk	6,2	1,68	1,0220	11,884
6	3,5% UHT milk	6,2	1,42	1,0252	13,104
7	3,7-4,1% pasteurized BIO milk	6,3	1,59	1,020	11,03

Determination of pH variation as a function of time and temperature

The following 3 experiments aimed to observe how the temperature or freshness of the samples influences the change in their acidity. Increased acidity is known to lead to curdling of milk, the separation of milk proteins. In fresh milk, this process naturally takes place very quickly.

Since our milk samples were commercial products, sterilized by different methods, we aimed to observe whether changes in milk acidity still occur under these conditions. To be able to follow how this phenomenon takes place, we chose to monitor the pH of milk samples kept at room temperature for 4 days, and then heated, at different temperatures, below the thermal coagulation limit of milk [2,7].

Experiment 1

Milk samples were stored at room temperature for 4 days and pH values were determined. The recorded results are given in Table 2 and Figure 3.

Experiment 2

On the 4th day the milk samples were stored at different temperatures for 5 minutes and the pH was monitored. The temperature was gradually increased from 25 ℃, to 40 ℃, to 60 ℃, respectively to 80 ℃. The recorded results are shown in Table 3 and Figure 4.

Experiment 3

The pH was measured in the milk samples heated at 80℃ after 5 minutes, 10, 15, 20 and respectively 25 minutes, and the data obtained can be noticed in Table 4 and Figure 5.

Table 2. Variation of pH in milk samples over 4 days stored at room temperature (21-24 ℃)

	Sample	pH			
		1st day	2nd day	3rd day	4th day
1	1,5% lactose-free milk	6,2	6,2	6,1	6,1
2	3,5% lactose-free milk	6,3	6,3	5,7	5,5
3	1,5% pasteurized milk	6,4	6,4	6,2	6,2
4	3,5% pasteurized milk	6,3	6,3	6,3	6,2
5	1,5% UHT milk	6,2	6,2	6,2	6,2
6	3,5% UHT milk	6,2	6,2	6,2	6,2
7	3,7-4,1% pasteurized BIO milk	6,3	6,3	6,2	6,1

Table 3. pH evolution with temperature for milk samples

	Sample	pH			
		25℃	40℃	60℃	80℃
1	1,5% lactose-free milk	6,1	6,1	6,0	5,9
2	3,5% lactose-free milk	5,5	5,3	5,1	5,0
3	1,5% pasteurized milk	6,2	6,2	6,1	5,9
4	3,5% pasteurized milk	6,2	6,2	6,1	6,0
5	1,5% UHT milk	6,2	6,2	6,1	6,0
6	3,5% UHT milk	6,2	6,2	6,1	6,0
7	3,7-4,1% pasteurized BIO milk	6,1	6,1	6,0	5,9

Table 4. Monitoring of pH variation over time in milk samples stored at 80℃

	Sample	pH				
		5 minutes	10 minutes	15 minutes	20 minutes	25 minutes
1	1,5% lactose-free milk	5,9	5,8	5,8	5,7	5,7
2	3,5% lactose-free milk	5,0	4,9	4,8	4,7	4,7
3	1,5% pasteurized milk	5,9	5,9	5,8	5,8	5,7
4	3,5% pasteurized milk	6,0	5,9	5,9	5,9	5,9
5	1,5% UHT milk	6,0	6,0	5,9	5,9	5,9
6	3,5% UHT milk	6,0	6,0	6,0	5,9	5,9
7	3,7-4,1% pasteurized BIO milk	5,9	5,8	5,7	5,7	5,6

RESULTS INTERPRETATION

The results obtained for casein concentration, pH, acidity and density are included in Figure 2. In this graph at once after opening the milk containers for all the analysed samples, the density and pH varied truly little from one sample to another. Moreover, the small density variations in the case of milk samples with 1.5% fat concentration compared to the same assortment with a 3.5% fat concentration did not follow a single rule, which shows the complexity of milk in terms of composition and that he is not just a simple emulsion of fats in aqueous solution. Only in lactose-free milk, the rule according to which fatter milk has a lower density was seen exactly. As expected, a lower density value was obtained for organic milk with the highest fat content, 3.7-4.1%.

Acidity also did not vary much from one milk sample to another; however, it had a slightly different evolution than the pH. The lowest acidity value was obtained for the 3.5% UHT sample, and the highest for the 3.5% pasteurized milk.

Regarding milk casein concentration, the lowest concentrations were obtained for lactose-free milk, showing that the removal of lactose, a disaccharide ($C_{12}H_{22}O_{11}$), also leads to a loss of milk casein. In contrast, for samples heat-treated either by pasteurization or UHT, the highest casein concentrations were obtained in samples with higher fat content, 3.5%.

Conversely, the casein concentration decreased in the organic milk samples, which shows us that the protein concentration also depends on the nutrient intake from the cows' diet.

As can be seen from the data recorded in Figure 3 and Table 2, the pH of milk stored at room temperature decreased progressively from day 1 to day 4, except for UHT milk where no change was observed.

The most substantial change in pH was seen in the 3.5% lactose-free milk sample, whose pH reached 5.5 on fourth day from 6.4 on first day.

Except for samples of UHT milk, the pH of milk samples started to decrease significantly from day 3, indicating the beginning of acid degradation for milk samples, curdling of milk.

In Figure 4 and Table 3, it is shown how the pH decreased with the increase in temperature in all categories of milk used in the experiment. The pH decrease was generally observed when the temperature increased above 60 °C. Minimum pH value was recorded at a temperature of 80°C and the maximum pH value at a temperature of 25°C for all analysed samples. Depending on the temperature, a greater decrease in pH was seen in 3.5% lactose-free milk. The other categories of milk used in this experiment recorded smaller decreases in pH with increasing temperature.

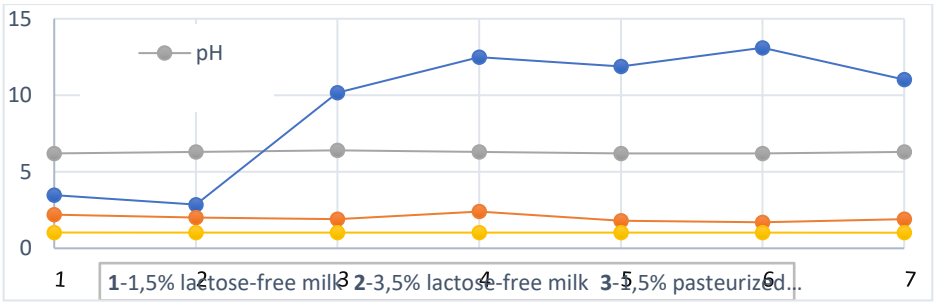

Figure 2. Variation of pH, acidity, density and casein concentration for different milk samples analyzed immediately after recipient opening at room temperature

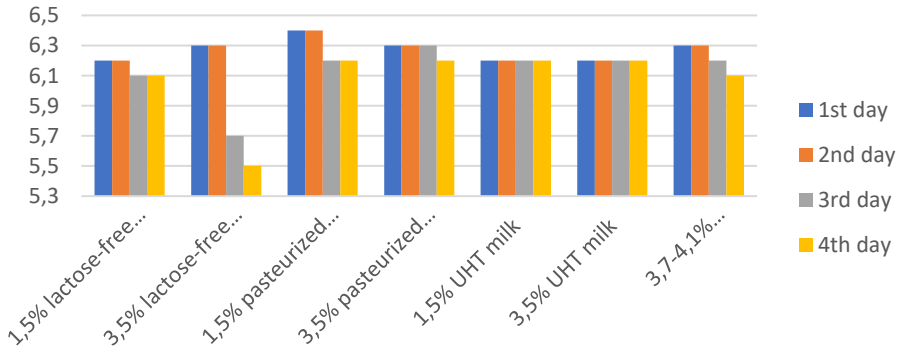

Figure 3. pH evolution in milk samples for 4 days at room temperature

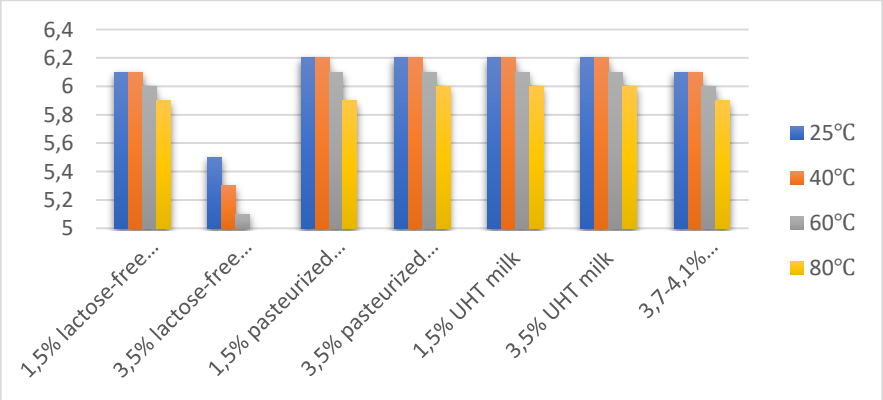

Figure 4. pH evolution with temperature for milk samples

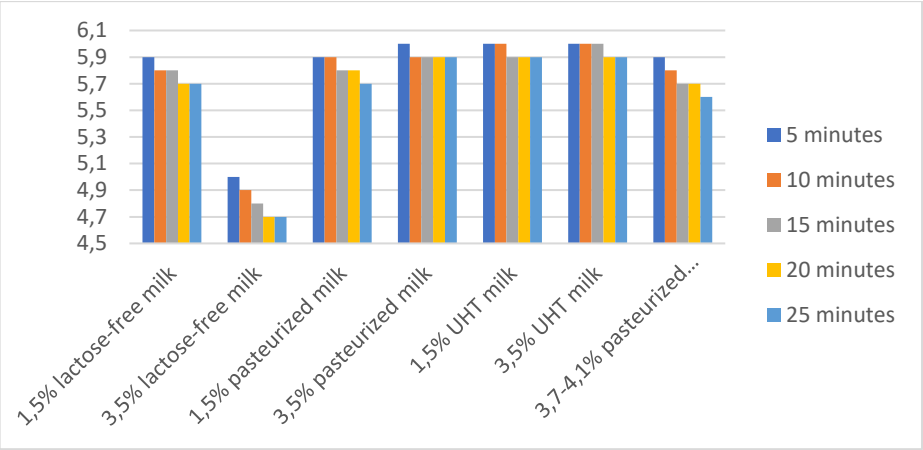

Figure 5. pH variation over time in milk samples stored at 80°C

In Figure 5 and Table 4, the pH of milk samples stored at 80°C starts to decrease after 10 minutes for all samples except UHT milk. In the 1.5% UHT milk sample, the pH drops after 15 minutes, and in the 3.5% UHT milk sample, the pH drops only after 20 minutes. Large decreases in pH over time at 80 °C were recorded for 3.5% lactose-free milk and 3.7-4.1% organic pasteurized milk. In both cases the decrease was 0.3 pH units.

CONCLUSIONS

The milk samples marketed in our region and studied in this work have remarkably similar pH, acidity and density parameters, characteristics of fresh milk samples kept under proper conditions, during the period of validity mentioned by the manufacturer. The pH of the milk samples ranged from 6.2 to 6.4, and the acidity ranged between 1.7 °T for the 3.5% UHT milk and 2.4 °T for the 3.5% pasteurized milk. The small density variations in milk samples with 1.5% fat concentration compared to the same variety with 3.5% fat concentration did not follow a unique rule due to the complexity of the sample composition. Regarding milk casein concentration, the lowest concentrations were obtained for lactose-free milk, and the highest casein concentrations were obtained in samples with higher fat content, 3.5%. the exception was organic milk, which shows that the nutrients in the cows' diet also play a significant role.

When storing the milk samples for 4 days at room temperature, only the UHT milk underwent insignificant changes in pH. None of the samples showed obvious signs of alteration, both altered appearance and smell. In the 3.5% lactose-free milk sample was noticed the most drastic pH decrease from 6.4 to 5.5. When heating the samples at different temperatures on the 4th day of storage at room temperature, it was found that the pH value decreases for most of the samples, and this diminution became more obvious above 60°C. The shape of the pH evolution curves is quite similar for studied samples. The largest decrease in pH was also recorded for 3.5% lactose-free milk, which shows that once the acid degradation process is triggered, it will go ahead faster, and the heat favours this process.

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Analele Universităţii din Oradea-Fascicula Chimie, Vol. XXIX (29), p.23-26, 2022

DATABASE WITH ECO-INHIBITORS OF CORROSION FOR CARBON STEEL IN ACID ENVIRONMENT

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Abstract. *Combating corrosion is justified by the need for safety in the operation of installations, the assurance of the appropriate quality of the manufactured product, limitation the material losses and pollution. Corrosion inhibitors are substances that, added in very small quantities, reduce, or even stop the corrosion phenomenon of metals. Some eco or green corrosion inhibitors are obtained from plant extracts. This paper presents the creation of a database with eco-inhibitors of corrosion obtained for plants, from literature database, a database that can be further supplemented with information from own studies or those of other researchers.*

Key words: corrosion, natural extracts, eco inhibitors, steel, acid environment

INTRODUCTION

The negative effects of corrosion are the losses of approximately 30% of the amount of metal consumed annually, to which are added additional expenses related to the shutdown of some installations to replace the corroded parts and last but not least the pollution with the various metals, which through corrosion, contaminates the water, the soil, or air [1,2].

The corrosion of metals and alloys is the process by which they are transformed from the state of zero oxidation, metallic, to a state of combined oxidation as a result of the interaction with substances in the environment.

Corrosion is a redox process that occurs on the metal/solution interface and is accompanied by electrons transfer, in two paired reactions; of metal oxidation and reduction of a chemical species from the environment.

Anti-corrosion protection can be done by several methods, therefore, the choice of the protection method is made by taking into account the maximum efficiency, right from the design phase. Protective coatings with metals, varnishes and paints are used especially in conditions of atmospheric corrosion. Treating the environment in order to reduce its aggressive nature, in many cases leads to a considerable decrease in the corrosion rate.

One of the methods to protect metallic materials against corrosion is by using corrosion inhibitors[1-3].

Corrosion inhibitors are organic substances, such as amines: pyridines, quinolines, thioureas, or inorganic: phosphates, chromates, that are adsorbed into the metal surface and considerably decrease the corrosion rate.

EXPERIMENTAL PART

Treating the environment in order to reduce its aggressive nature leads in many cases to a considerable decrease in the corrosion rate. One of the methods to protect metallic materials against corrosion is the use of corrosion inhibitors[1-3].

Corrosion inhibitors are organic substances, such as amines: pyridines, quinolines, thioureas, or inorganic: phosphates, chromates, that are adsorbed into the metal surface and considerably decrease the corrosion rate.

Green corrosion inhibitors or eco-inhibitors are biodegradable and do not contain heavy metals or other toxic compounds [2].

Like regular corrosion inhibitors, eco-inhibitors have the same classifications but are derived from natural organic and/or biological sources unlike the synthetic ones, and for inorganic sources, do not contain heavy metals.

Eco or green corrosion inhibitors are part of the following categories of substances: plant extracts, drugs, amino acids, surfactants, biopolymers and ionic liquids [4].

In order to have a broader picture of the extracts with natural corrosion inhibitors used up to now for steel in an acid environment, a database has been created with natural extracts prepared from plants, reported in the literature database. The database initially includes 20 extracts, used as corrosion inhibitors of carbon steel in an acid environment, and it will be supplemented both with the results of the present study and with the results of other researchers.

The database, table 1, includes the following categories of data: the name of the plant, in Romanian and Latin, the parts of the plant from which the extract was made, the classes of substances that are found in the largest quantity, identified as being part of the natural extract, the environment in which the anticorrosive effect of the natural eco-inhibitors was identified, - the concentrations of the eco-inhibitor in the prepared solutions, interval and optimal value, the maximum efficiency of the inhibitor.

Table1 is just a part of the database and it is given as example.

Eco-inhibitors or green organic inhibitors according to their natural compounds or the products they contain, such as amino acids, alkaloids, phenols and polyphenols, fatty acids, or as biological (chitosan, amino acids, bacteria and fungi), vegetable (plants). extracts, shells, tannins) and pharmaceutical drugs [5-16].

Table 1. Database of corrosion eco inhibitors for steel in acid environment, extracted from plants.

Nr.	Den romana	Den Latina	Metal	Media	Solution	Maximum Eficiency	Interval conc
1	Usturoi	Allium Sativum	carbon steel	1N H2SO4	50% vol in ethylic alcool	70,66% in the presence of 2% vol	0.1, 0.2, 1 and 2 % volume
2	Paciuli	Pogostemon Cablin	carbon steel	1N H2SO4	oily extract	66,31% in the presence of 2% vol	0.1, 0.2, 1 and 2 % volume
3	Nuc	Juglans Regia	carbon steel	1N H2SO4	50% vol in ethylic alcool	50,12% in the presence of 1% vol	0.1, 0.2, 1 and 2 % volume
4	Fag	Fagus Sylvatica	carbon steel	1N H2SO4	75% vol in ethylic alcool	69,47% in the presence of 0.05% vol	0.05, 0.1, 0.2, 1 and 2 % volume
5	Argan spinos	Argania spinosa	Mild steel	1.0 M HCl	1g powder in 100 ml HCl	97% at 3 g/L	0,05, 0,1, 0,5, 1, 1,5, 2, 2,5, 3
6	Argan spinos	Argania spinosa	Mild steel	1.0 M HCl	oil g /L	91% at 6 g/L	0,1, 0,5, 1 2, 3, 4, 5, 6 g/L
7	Mahon din Senegal	Khaya senegalensis	Carbon steel	1.0 M HCl	Acid aqueous extract, t. 10.0 g of powdered dry leaves were refluxed in 500 mL of 1.0 M HCl	91,10%	0,25, 0,5, 1, 2 g/ L
8	prunul de cafea indian sau	Coffe plum, Flacourtia jangomas	Mild Steel	1M HCl	10 g of dried powder of plant were digested in 200 mL 1M HCl	leaf extract 98.1%, root 97.3 % , stem bark 96.4% at 5% in HCl	0,5, 1, 1, 5%

9	prunul de cafea indian	Coffe plum, Flacourtia	Mild Steel	1N H2SO4, 0,5M H2SO4	x	leaf extract 95.8%, root 91.2 % , stem bark	0,5, 1, 1, 5%
10	iedera diavolului	Epipremnum aureum	Low-carbon steel	1M H2SO4	150 g powder in 1L of solvent [methanol (MeOH), ethanol (EtOH), ethyl acetate (EtAC)]	92,37% la o conc de 600 ppm	(100–600ppm) for 3h
11	x	tara	Mild steel	0.5 M HCl	4 g/L tannin	81,25-96%	x
12	acacia neagra,	Acacia mearnsii	Mild steel	0.5 M HCl	4 g/L tannin	75.85%	x
13	bayberry	Myrica pensylvanica	Mild steel	0.5 M HCl	4 g/L tannin	72.69%	x
14	lavandula franceza	Lavandula Stoechas	carbon steel	1M HCl	L. stoechas oil	61,97% la 2 g/L	0,25, 0,5, 1, 1,5 ,2g/l
15	liliac indian	Azadirachta indica	mild steel, Al,	HCl, H2SO4	extract	x	x
16	Hibiscus	Hibiscus rosa-sinensis	mild steel	1 M HCl	extract	x	x
17	Portocala	Essential oil · Orange zest	mild steel	1M HCl	orange Essential oil	75.45% at 2.5 g /L	0,5, 1, 1,5 , 2, 2,5 g/L

CONCLUSIONS

Following the research carried out so far on corrosion eco inhibitors for metals and alloys, in aqueous environments, the following conclusions can be drawn:

- Regardless of classification, nearly 80% of green organic corrosion inhibitors are categorized as mixed inhibitors, which shield the metal from corrosion by forming a coating through chemical and physical adsorption [3].
- The current state of knowledge indicates a huge potential for the exploitation of natural, non-toxic, environmentally friendly extracts, with a corrosion inhibitor effect, which can be used for the anti-corrosion protection of various metals and alloys, in different solutions or aqueous environments.

The studies carried out until now focused on the evaluation of the behavior of the most common metals and alloys: steel, aluminum, copper, copper, aluminum and magnesium alloys, which were studied in different aqueous environments: acidic, neutral or basic. Although in the specialized literature these studies have been done for more than 10 years, the multitude of plant specimens means that these studies are just at the beginning.

Funding: This research was funded by the University of Oradea, within the Grant Competition "Scientific Research of excellence related to priority fields with capitalization through Transfer technology: INO-TRANSFER-UO", Project No. 327/21/12/2021.

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DETERMINATION OF ANTIOXIDANTS FROM FOENICULUM VULGARE AND CUMINUM CYMINUM SEEDS

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Abstract

In this work we aimed to determine the antioxidant composition of the seeds and bulb with edible leaves of fennel (Foeniculum vulgare) and cumin seeds (Cuminum cyminum) using different methods. Hydroalcoholic extracts have been prepared from both seeds and the leafy stem of fennel, respectively from cumin seeds. From these extracts, the polyphenol content was determined using the Folin Ciocalteu quantitative method. The determination of the composition in antioxidants was made by reverse phase liquid chromatography with mobile phase B-Acetonitrile acidified with Formic Acid and A-Acidified water with Formic Acid, at pH 3. The detectors used were a mass spectrometer and a UV spectrophotometer. The antioxidant capacity was determined by the CUPRAC method, in which the reducing activity of antioxidants on Cu(II) ions at Cu(I) revealed by the formation of a complex with an adequate ligand was also determined spectrophotometrically.

Key words: *chromatography, polyphenols, antioxidant activity, spectrophotometry, seed*

INTRODUCTION

The fennel plant (*Foeniculum vulgare*) is part of the *Apiaceae* family, being consider a medicinal plant native to the Mediterranean region, which grows in the central European area but also in the other warm regions of Europe [1]. Fennel seeds have a similar flavor to anise and are used as condiment, and in Mediterranean cuisine bulbs and leaves are used, both raw and cooked, in salads, with pasta, vegetable dishes [1-7]. It was identified that methanolic extract from *Foeniculum vulgare* fruits exhibit antioxidant activity and *Foeniculum vulgare* acetone essential oil extracts exhibit a strong antioxidant activity compared to butylate hydroxyanisole (BHA) and butylate hydroxytoluene (BHT) [9]. The main component of *Foeniculum vulgare*, anethole, has antithrombotic activity due to vasorelaxant action with significant protection from gastric damage. *Foeniculum vulgare* preparations have also been shown to have anti-inflammatory, estrogenic, hepatoprotective and antimicrobial effects [10-13].

Cuminum cyminum (cumin) is an aromatic herb cultivated in the Middle East, India, China and several Mediterranean countries, including Tunisia [14]. The seeds of *Cuminum cyminum* have in their composition an essential oil, in the proportion of 2.5-4%. *Cuminum cyminum* essential oil has in its composition [18]: 25-35% *cuminaldehyde*, 28-30% α and β *pineene* and 22% *limonene*. Cumin is a traditional spice, very used since the Middle Ages [15]. It is a major constituent of curry powder and is the second most widely used spice, after pepper [16]. *Cuminum cyminum* oil is recognized for showing antibacterial activity. The mode of action of cumin oil is to damage the cell membrane and the release of DNA and intracellular proteins [17, 18]. *Cuminum cyminum* oil plays an important role in treating rheumatic conditions, treating disorders of the digestive and bronchopulmonary systems, helps to soothe cough and to treat hoarseness [19]. The seeds are used for diuretic, stimulating and astringent properties [20, 21].

Hydroalcoholic extracts have been prepared from both seeds and the leafy stem of fennel, respectively from cumin seeds. From these extracts, the polyphenol content was determined using the Folin Ciocalteu quantitative method. The determination of the composition in antioxidants was made by reverse phase liquid chromatography with mobile phase B-Acetonitrile acidified with

Formic Acid and A-Acidified water with Formic Acid, at pH 3. The detectors used were a mass spectrometer and a UV spectrophotometer. The antioxidant capacity was determined by the CUPRAC method, in which the reducing activity of antioxidants on Cu(II) ions at Cu(I) revealed by the formation of a complex with an adequate ligand was also determined spectrophotometrically. The data obtained were analyzed and discussed comparatively.

MATERIALS AND METHODS

The apparatus used were a Grindomix Retsch GM 200 grinding mill, a Kern ALJ 210 analytical balance, Heidolph Unimax 1010 Shaker. For the analysis of the extracts, we used UV-VIS 250 Spectrometer from Analytic Jena and a drying stove. The chromatographic analysis was performed with a Shimadzu LC-MS device equipped with SPD-10A UV detector. The reagents used were of high analytical purity.

Samples preparation

For the preparation of the extracts, we chose organic seeds of *Foeniculum vulgare* and seeds of *Cuminum cyminum* from various specialized traders. For this study, the samples chosen were from the seeds of and *Cuminum cyminum*, and from the fresh plant of *Foeniculum vulgare* were taken the bulb and leaves.

The green leaves of *Foeniculum vulgare* were kept at room temperature until dry, after which the sterilization and grinding stages took place. Foeniculum vulgare bulb was cut into small pieces and shredded. The seeds of Foeniculum vulgare and the seeds of Cuminum cyminum were ground into a fine powder. A hydroalcoholic solution was prepared, from ethyl alcohol and distilled water in a 1:1 volume ratio.

1 g of each sample was extracted in 10 mL hydroalcoholic solution for 60 minutes at 200 spin in the Heidolph Unimax 1010 Shaker. The resulting samples were filtered and stored in plastic containers in the refrigerator at an appropriate temperature without affecting the properties of the plants analyzed.

Determination of total polyphenol content

Polyphenolic compounds are biosynthesized in plants as secondary metabolites, with an important role in the sensory and nutritional quality of fruits, vegetables and plants. Polyphenols are among the most studied phytochemicals, mainly due to their antioxidant activity, their bioactive functions and actions as biostimulators or growth inhibitors in plants [22,23]. Polyphenols were found using the Folin Ciocalteu method [24]. After 35 minutes of incubation at 50 °C in the drying stove, the absorbance was read at 750 nm, at the UV-VIS Specord 250 spectrophotometer [25]. The calibration curve used to interpret the results obtained is one for GA gallic acid solutions in the concentration range 5 to 250 ug/ml [26].

Chromatographic determination of polyphenol types

In order to determine the nature of the polyphenols in the composition of the studied samples, we used the reverse phase chromatographic method, with the non-polar stationary phase and with the polar mobile phase, observing the two conditions of the mobile phase in which B is Acetonitrile acidified with Formic Acid and A is acidified water with Formic Acid, at pH 3. The gradient program used was with a time of 55-60 min 95% B, 5-55 min for 40-95% B, 20.01-50 min for 5-40% B, 0.01-20 min. for 5% B. Rate of solvent flow used was 0.2 ml/minute at a temperature of 20 °C. The measurements were performed at 280 nm and 320 nm wavelengths. Calibration curves for the identified species for which standards were achieved in the concentration range of 20 to 55 µg/ml. The results obtained were expressed in µg/g.

Determination of antioxidant capacity

The cupric reducing antioxidant capacity (CUPRAC) causes an increase in absorbance at a certain prespecified wavelength, after the antioxidant reacts with the chromogenic reagent, namely it reduces iron and copper ions at lower valences (Fe(II) and Cu(I)), and further they form load transfer complexes with appropriate ligands. The advantage of using the CUPRAC method is that the CUPRAC reagent (electronic transfer agent of the outer sphere) is fast enough to oxidize thiol antioxidants.

The CUPRAC method of measuring antioxidant capacity is based on absorbance measurement at 450 nm after 30 minutes of reaction.

The reference substance used is Trolox, a both fat-soluble and water-soluble antioxidant that mimics the structure of vitamin E. Molar absorptivity coefficient for Trolox using the CUPRAC method is ε =

1.67x104 $L \cdot mol^{-1} \cdot cm^{-1}$ [25]. The results are expressed in mM Trolox/g dry matter. When the formation of the load transfer complex Cu(l)-Nc occurs, the transition to orange staining occurs.

RESULTS AND DISCUSSIONS

Total polyphenol content from Foeniculum vulgare and Cuminum cyminum

In Table 1 and Figure 1 can be seen the results obtained for the alcoholic extracts.

Table 1. Total polyphenol content value

Extract	Total polyphenols (mg GAE/100 g)
Foeniculum vulgare seeds	2.76
Foeniculum vulgare leaves	0.62
Foeniculum vulgare bulb	0.58
Cuminum cyminum seeds	1.63

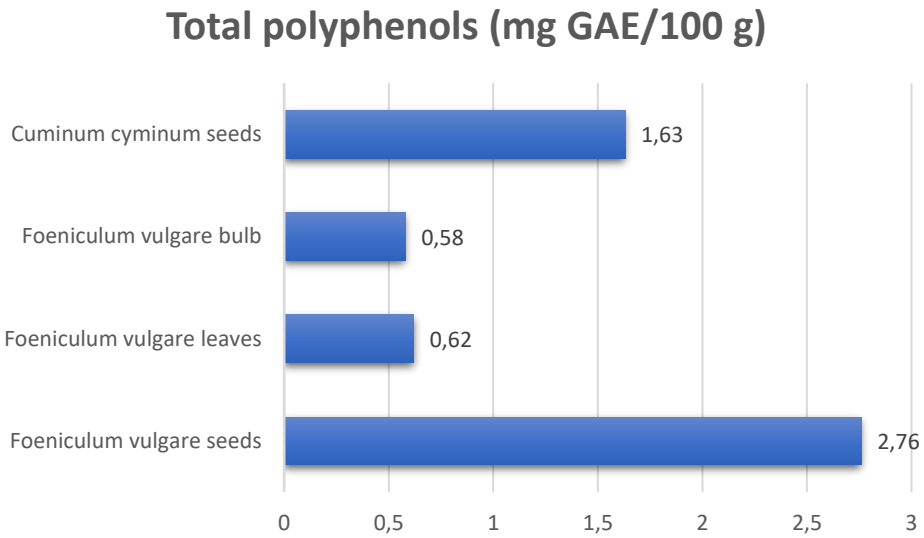

Figure 1. Total polyphenol content of *Foeniculum vulgare* and C*uminum cyminum* extracts

As can be seen in the fig. 1, the highest total polyphenol content of the extracts is in *Foeniculum vulgare* seeds of 2,76 mg GAE/100 g, followed by the extract from the seeds of *Cuminum cyminum* which reported a value of 1,63 mg GAE/100 g. Significant differences between the result obtained from the extract of *Foeniculum vulgare* seeds to the vegetative part with a lower content for these last were observed, i.e., 0,62 mg GAE/100 g for bulb and 0,58 mg GAE/100 g for leaves.

The studies conducted by other researchers recorded related results with ours, so 18.6 mg GAE/g dry matter obtained for the seeds of Tunisian *Cuminum cyminum* [26]. Higher concentrations of total polyphenols found in the seeds extracts of the two types of samples analyzed compared to the green plant parts of *Foeniculum vulgare* analyzed, and of these, the largest amounts of polyphenols found in the seeds of *Foeniculum vulgare.*

The results of the chromatographic analysis of polyphenols

In Tables 2 and 3, respectively in Figures 2 and 3, the individual polyphenolic profile is shown for the analyzed plants of the *Apiaceae* family, namely for *Foeniculum vulgare* and *Cuminum cyminum* obtained following the chromatographic separation with mobile phase of B- Acetonitrile acidified with Formic Acid, A- Acidified water with Formic Acid, at pH 3 of the hydroalcoholic extracts from the seeds of plants.

With the LC-MS method, 11 different polyphenolic compounds have been identified in the extract of *Foeniculum vulgare* seeds (Table 2). The LC-MS profile of the seed extract reveals an important content of Rosmarinic Acid (2231.86 µg/g), Kaempferol (1780.58 µg/g), Epicatechin (1443.21 µg/g), Rutin (698.75 µg/g). Slightly lower amounts of Resveratrol (215.80 µg/g), Gallic Acid (184.06 µg/g) and very small amounts of Ferulic Acid (19 µg/g) and Coumaric Acid (32 µg/g) were obtained.

With the LC-MS method, 11 different polyphenolic compounds have been identified in the extract of *Cuminum cyminum* seeds (Table 3). A rich content of Kaempferol (247.32 µg/g), Gallic Acid (83.01 µg/g), Resveratrol (70.02 µg/g), as well as smaller amounts of Epicatechin (50.37 µg/g) and Quercetin (42.85 µg) can be observed /g). Ferulic acid in this case is almost non-existent, and Protocatechuic acid, respectively Coumaric acid are both in tiny amounts around 6-7 µg /g.

Table 2. Individual polyphenolic content for *Foeniculum vulgare* seeds extract

Polyphenols	Retention time (min)	(M/Z)	Concentration (µg/g)
Rosmarinic acid (RO)	29.8	369	2251.67
Routine (RU)	26.1	611	698.75
Ferulic acid (FE)	25.1	198	19.12
Coumaric acid (CU)	24.9	167	31.82
Epicatechin (EC)	22.9	293	1443.21
Caffeic acid (CA)	22.1	183	100.89
Protocatechuic acid (PR)	10.9	155	61.80
Gallic acid (GA)	4.9	173	184.06
Resveratrol (RS)	32.1	229	215.80
Quercetin (QU)	32.8	305	571.80
Kaempferol (KP)	35.3	287	1278.07

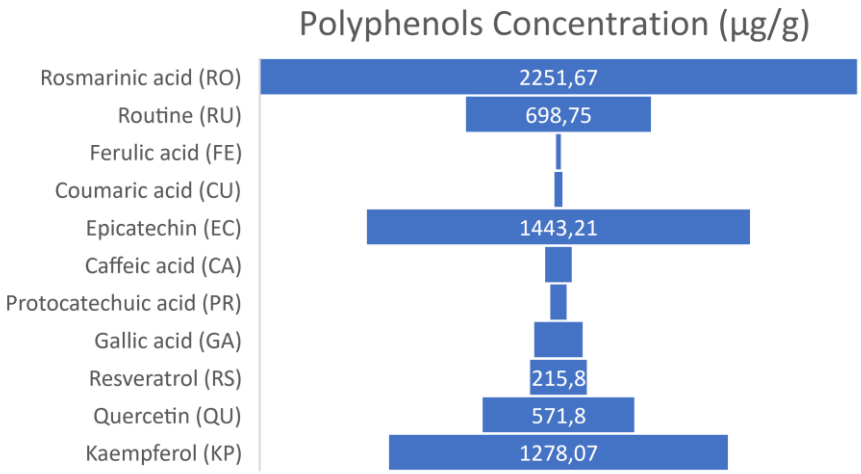

Figure 2. LC-MS profile for Foeniculum vulgare seeds

Table 3. The individual polyphenolic content for the *Cuminum cyminum* seeds extract

Polyphenols	Retention time (min)	(M/Z)	Polyphenols concentration (µg/g)
Kaempferol (KP)	35.2	287	247.32
Gallic acid (GA)	4.9	171	83.01
Rosmarinic acid (RO)	29.1	363	62.86
Resveratrol (RS)	32.2	229	70.02
Routine (RU)	25.9	611	58.80
Epicatechin (EC)	22.9	292	50.37
Quercetin (QU)	32.3	303	42.85
Caffeic acid (CA)	22.2	182	18.05
Protocatechuic acid (PR)	10.9	155	6.81
Coumaric acid (CU)	24.7	166	6.19
Ferulic acid (FE)	24.9	196	0.47

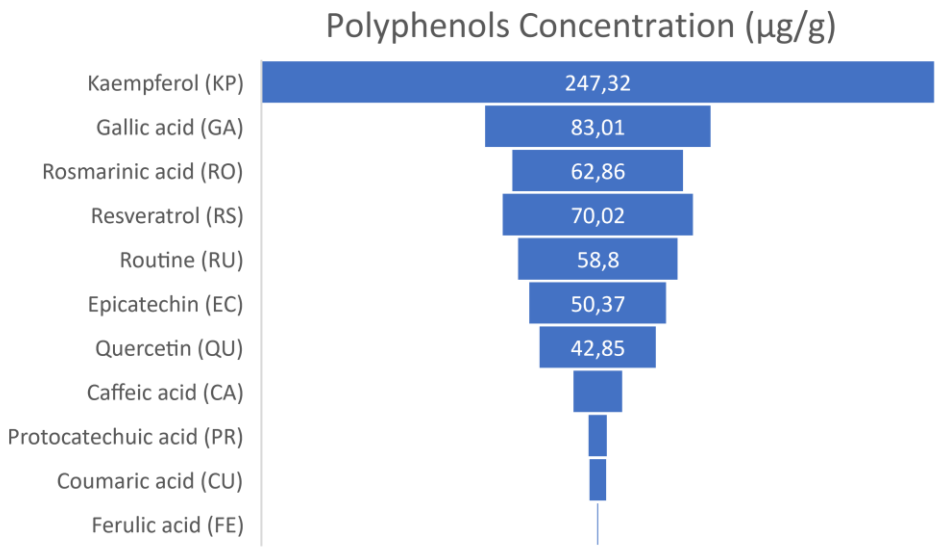

Figure 3. LC-MS profile for *Cuminum cyminum* seeds

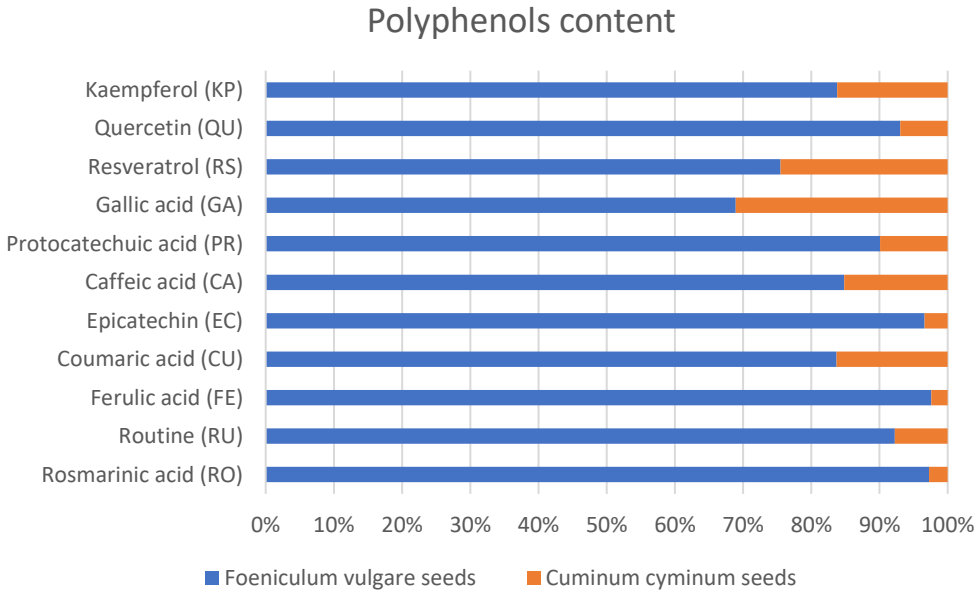

Figure 4. Comparative LC-MS profile for *Foeniculum vulgare* seeds and *Cuminum cyminum* seeds

In Figure 4 we can see the presence of 11 individual polyphenols in the seeds of *Foeniculum vulgare* and seeds of *Cuminum cyminum*. Following the LC-MS profile we can find that the predominant is Gallic Acid, Kaempferol, Rosmarinic Acid and Epicatechin. Analyzing the values obtained we can find that a rich content of Kaempferol (1278.07µg /g) is found in the seeds of *Foeniculum vulgare*, compared to the seeds of *Cuminum cyminum* (247.32 µg /g), with a much lower content.

In the *Foeniculum vulgare* seed extract the predominant is Rosmarinic Acid (2251.67 µg/g), Kaempferol (1278.07 µg/g) and Epicatechin (1443.21 µg/g) and in the *Cuminum cyminum* seed extract the predominant is Kaempferol (247.32 µg/g), Gallic acid (83.01 µg/g) and Resveratrol (70.02 µg/g).

The two analyzed seed samples, fennel and cumin, have in common high concentrations of Kaempferol and Rosmarinic Acid, medium concentrations of Resveratrol and Rutin and very low concentrations of Caffeic Acid, Protocatechuic Acid, Coumaric Acid and Ferulic Acid.

Antioxidant capacity

As we can see in Table 4, the results obtained for the plant extracts of *Foeniculum vulgare*-seeds, *Foeniculum vulgare*-leaves and *Cuminum cyminum*-seeds are highlighted.

Table 4. Results of antioxidant capacity

Sample	Concentration (mg Trolox/L)
Foeniculum vulgare-seeds	3379.61
Foeniculum vulgare-leaves	1432.82
Cuminum cyminum-seeds	*867.04*

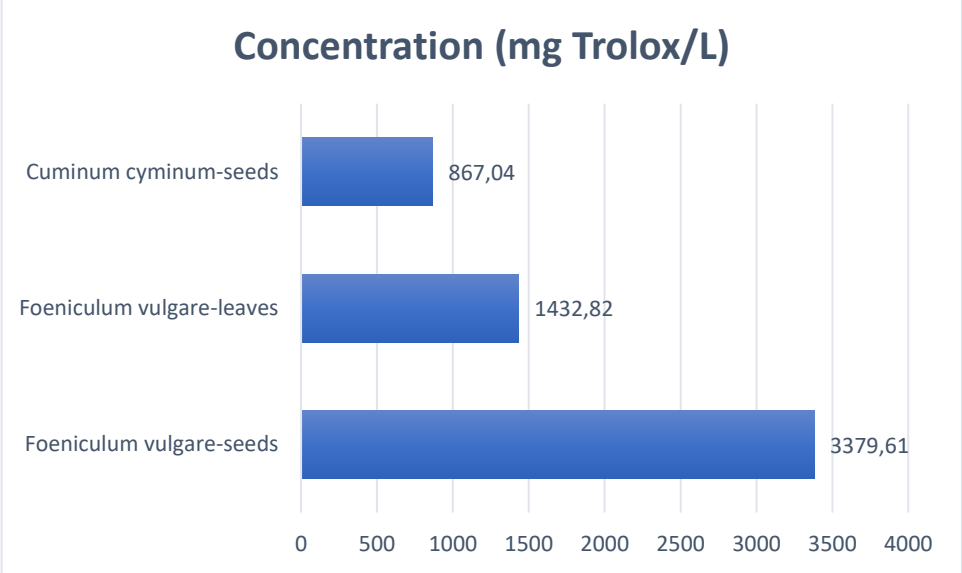

Figure 5. Antioxidant capacity values of *Foeniculum vulgare* (seeds and leaves) and *Cuminum cyminum* seeds

As we can see from Figure 5, *Foeniculum vulgare* seeds have the highest antioxidant capacity (3379.61 mg Trolox/L). The obtained results correlate very well with the results obtained by the Folin Ciocalteu method.

CONCLUSIONS

Analyzing the total content of polyphenols in *Foeniculum vulgare* seeds, *Foeniculum vulgare* leaves, *Foeniculum vulgare* bulb and *Cuminum cyminum* seeds, we found that the highest values were reported in the case of seed extracts, respectively we obtained for *Foeniculum vulgare* seeds 2.76 mg GAE/100 g.

Following the LC-MS profile we found that a high content of Kaempferol (1278.07µg / g) is found in *Foeniculum vulgare* seeds, while in the case of *Cuminum cyminum* seeds we obtained a much lower value, namely (247.32 µg /g). Rosmarinic acid is found in a large amount in *Foeniculum vulgare* (2251.67 µg / g) being the majority, followed by Kaempferol. At an overview of the results obtained from the chromatographic analysis with an LC-MS device, the fennel and cumin seed samples analyzed showed high concentrations of Kaempferol and Rosmarinic Acid, medium concentrations of Resveratrol and Rutin, and very low concentrations of Caffeic Acid, Protocatechuic Acid, Coumaric Acid and Ferulic Acid.

Analyzing the obtained values for the antioxidant capacity of *Foeniculum vulgare* seeds, *Foeniculum vulgare* leaves and *Cuminum cyminum* seeds, we can also notice that *Foeniculum vulgare* seeds have the greatest capacity, 3379.61 mg Trolox/L.

All three methods for the determination of polyphenols with the Folin Ciocalteu method, the composition in antioxidants by liquid chromatography associated with mass spectrometry (LC-MS method) and the determination of antioxidant activity by the CUPRAC method have shown that the seeds of *Foeniculum vulgare* are richer in antioxidants and more effective in terms of antioxidative activity than the seeds of *Cuminum cyminum*. Also, the results obtained by the Folin Ciocalteu method showed that the seeds, compared to other parts of the plant (bulb, leaves) have a greater load in antioxidants.

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THE SEPARATION OF CAROTENOIDS FROM NETTLE LEAVES (*URTICA DIOICA)* BY TLC

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Abstract: *Carotenoids are plant pigments spread in plant tissues and animals. These pigments print yellow, red or blue color, tissues that were. In herbal the carotenoids was in leaves, fruits, stems, roots, seeds, petals and in pollen. Amount of carotenoids in dried leaves is between 250-650 mg/g. Carotenoids pigments are synthesized only of vegetable. Carotenoids have practical applications in medicine and cosmetics and their use as food additives, dyes and antioxidants.*
Key words: *carotenoids, β-caroten,xantofila,licopen,violaxantina , TLC, Silica gel (SilG, Merk).*

1.INTRODUCTION

Carotenoids along with flavonoids and isoflavones are biological substances, called phytochemicals („phyto" means „from herbals") which give the flavor, aroma and resistance to the disease having an important role in photosynthesis and photoprotection . Food consumption rich in β – criptoxantina, carotenoid a color orange – red significantly decreases risk of lung cancer, according to a study published in 2003 in „Cancer Epidemiology, Biomarkers and Prevention"magazine[1] .

Analysis of carotenoids from plants was inserted by Tswett (1906), subsequent findings, identification and elucidation
structure have been developed by Karrer, Zechmeister, Talerand, Goodwiszi [2-5]. Curent methods for operation and analysis carotenoids includes new methods separation as TLC and HPLC to replace or complementary with classical technique (locked column chromatography) [6-8]. There is limited on composition carotenoids from the nettle leaves separated by open column chromatography. Purpose of this study is characterizing the type of carotenoids from nettle leaves using methods of separation (TLC, HPLC) to identify major components.The leaves contain carotenoids (beta-carotene in a percentage of 0.02 - 0.04%; xanthophyll; lycopene; violaxanthin), protoporphyrins (chlorophyll A and B), vitamins (B2, C, K, folic and pantothenic acid), proteins (23 – 24%), amine derivatives (histamine, acetylcholine, serotonin), organic acids (acetic, butyric, caffeic, chlorogenic, ferulic, formic, sinapic), flavones (3-0-glucosides and 3-0-rutinosides of kaempferol, quercetol, isorhamnetol), catechin tannins, coumarins (scopoletol), free and glycosidated beta-sitosterol, volatile oil, mineral substances (silicon dioxide, calcium oxide, magnesium oxide, iron oxide).

2.EXPERIMENTAL

Solvents used in chromatographic separation are of analytical purity (methyl acetate, hexane, chloroform, acetonitrile) or distilled (acetone, ethyl ether, ethanol) and as buffer substances use : TEA and antioxidants (butylated hidroxytoluene – BHT).

The nettle leaves were dried at room temperature in airflow about 6 – 7 days, have shelled and after which the maceration. Seeds macerated dry in oven about 2 hours, then are crushed in mortar to reach as a powder. The powder to be extracted with chloroform : methanol : petroleum ether (2 : 3 : 1 v/v/v) for 8 hours at dark to prevent degradation and oxidation of carotenoids. Crude extract filtered, evaporate to drying in Rotovapor and re – suspended in ethyl ether.

Saponification was done in ether being added to 15% KOH in ethanol until the final concentration of 5 % V/V KOH. Mixture was stirred with a magnetic stirrer for 4 hours, carotenoids being extracts for 2 – 3 times successively with ethyl ether a solution with neutral pH.

Soap is removed, extract all the carotenoids was evaporated with Rotovapor, brought to a certain volume and used for chromatographic separation.

The total concentration of carotenoids of total extract (TE) is calculated by comparing absorbance A (α_{max} = 420 nm) with a specific absorbing (α = 400 nm).

$$X = (A \times Y \times 1000) : (2500 \times 100) = A \times Y / 250 \quad (1)$$

Where:
X – weight carotenoids of sample (mg)
Y – sample volume (ml)

To characterize the composition in carotenoids of nettle leaves has made a preliminary separation total extract (TE) on a column of alumina (Al_2O_3 with 5% water). Small volumes were taken of total extract and were placed on the column and eluted with 3 solvent systems:

- 100% petroleum ether (fraction 1)
- petroleum ether : ethyl ether (2 : 1 v/v) (fraction 2)
- methanol in ethyl ether 10 % v/v (fraction 3)

3.RESULTS AND DISCUSSIONS

Total extract has undergone quantitative analysis. Peakurile obtained of densitograma TLC (figure 1) recorded at 450 nm have been identified and compared with total extract, with separation standard (S) same conditions. Major carotenoids

Total extract and fractions 1 – 3 released from the alumina column were separated on silica gel plates (SilG, Merk) and chromatograms were analyzed in Shimadzu CS – 5000 with beam with dual wavelength. For total extract used a separation of two stages:

- with 15 % v/v acetone in petroleum ether
- with 100% petroleum ether

For fractions 1 – 3 developing systems was:

- 10 % v/v acetone in petroleum ether (fraction 1)
- 15 % v/v acetone in petroleum ether (fraction 2)
- 20 % v/v acetone in petroleum ether (fraction 3)

identified were: β-caroten, xantofila, licopen, violaxantina

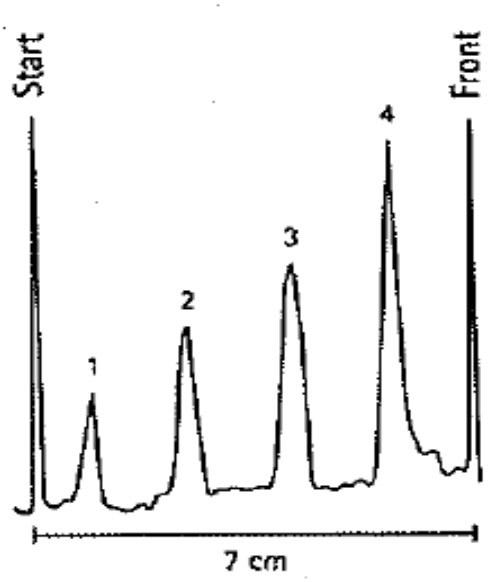

Figure 1. TLC chromatogram the total extract: 1-β-caroten; 2- licopen; 3- violaxantina; 4- xantofila

So we learned only percentage composition the main carotenoids in extract and not in their absolute: 26,46% β-caroten; 21,30% licopen, 31,25% violaxantina, 40,15% xantofila. Fractions 1 collected from alumina columns were analyzed by TLC.

Fraction 1 contains 2 main peakuri results of TLC separation representing non – polar carotenoids (licopen and xantofila).

4.CONCLUSIONS

Total amount of carotenoids from nettle leaves assessed by spectrometry in the VIS the total extract was 85 mlg^{-1} dry mass. By chromatographic analysis (TLC) have highlighted the main carotenoids present in nettle leaves,β-caroten, xantofila, licopen, violaxantina in total extract distributed differently between fractions diluted of alumina column. TLC proved a rapid and useful method for separation of carotenoids from nettle leaves.

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(12pt)

INSTRUCTIONS FOR AUTHORS (TIMES 14 PT BOLD, CAPITAL LETTERS, CENTRED)

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First name SURNAME[1], First name SURNAME[2] (12 pt bold)

[1]Affiliations and addresses (12 pt)

(12pt)

Abstract: *Abstract of 50-120 words (12 pt italic). It contains concise information about: objectives of the work, the results obtained, conclusions*

Key words: List 2-6 keywords. (l0 pt, italic).

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The paper has to be written in English. Each paper should be concise including text, figures and tables. Authors are kindly requested to submit in electronic format, Microsoft Word file form, 2003, 2007, 2010. The suggested structure of the main text: Introduction; Methods and Materials, Results and Discussions; Conclusions; References. (12pt)

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Tables, together with figures should be placed in their order of appearance in the text and numbered consecutively. Table captions containing the number of the table, and should be placed above the table. Tables should be clearly captioned and all symbols should be properly explained in either the table or its caption.

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